

Applicant : Shih-Jen Liu, et al
Serial No. : 10/072,185
Filed : February 8, 2002
Page : 5

Attorney's Docket No.: 13886-002001 / 01P0325

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 5, line 8, has been amended as follows:

Hsp70 gene was amplified from human hepatocellular carcinoma HepG2 cDNA with a gene-specific forward primer: 5'-cgccggatccATGGCCAAAGCCGC~~GGC~~-3' (SEQ ID NO:1), and a gene-specific reverse primer: 5'-cgccggatccCTAATCTACCTCCTCAATGG-3' (SEQ ID NO:2). The 1.92 kb Hsp DNA fragment was cleaved with *BamHI* and ligated with a *BamHI*-cleaved pRSETA vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp70 gene was named as pRSETA/Hsp70.

Paragraph beginning at page 5, line 16, has been amended as follows:

Hsp C-terminal DNA fragment was amplified from pRSETA/Hsp70 with an HspC'-specific forward primer: 5'-ggaaattcGCGATGCCAACGGCATCCTGAAC-3' (SEQ ID NO:3) and an HspC'-specific reverse primer: 5'-ggaaatttCTAATCTACCTCCTCAATGGTG-3' (SEQ ID NO:4). The 0.5 kb HspC' DNA fragment was cleaved with *ApoI* and ligated with an *EcoRI*-cleaved pRSET vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing Hsp C-terminal DNA fragment was named as pRSET/HspC', which served as a backbone for construction of tumor antigen-HspC' expression plasmids.

Paragraph beginning at page 5, line 26, has been amended as follows:

HepG2 cells were homogenized in RNAzol™ B solution, and total RNA was prepared according to the protocol provided with the kit. The cDNA was synthesized by SuperScript™ II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. AFP gene was amplified from HepG2 cDNA with a gene-specific forward primer: 5'-gccccatccACACTGCATAGAAATG AATATG-3' (SEQ ID NO:5), and a gene-specific reverse primer: 5'-gccccatccAACTCCCAAAG CAGCACGAG-3' (SEQ ID NO:6). The 1.77 kb AFP DNA fragment was cleaved with *BamHI*.

Applicant : Shih-Jen Liu, et al
Serial No. : 10/072,185
Filed : February 8, 2002
Page : 6

Attorney's Docket No.: 13886-002001 / 01P0325

and ligated with a *BamHI*-cleaved pcDNA3 vector. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing AFP gene was named as pcDNA3/AFP.

Paragraph beginning at page 6, line 17, has been amended as follows:

Total RNA was prepared from LNCaP cells with RNAzolTMB (Tel-Test). LNCaP cDNA was synthesized by SuperScriptTM II Reverse Transcriptase (GIBCO BRL) with an oligo-d(T)₁₂₋₁₈ primer. PSA gene was amplified from LNCaP cDNA with a gene-specific forward primer: 5'-ATGTGGGAGGCTGGAGTG-3' (SEQ ID NO:7) and a gene-specific reverse primer: 5'-GGGGTTGCCACGATGGTG-3' (SEQ ID NO:8). The PCR reaction was performed by DyNAzymeTM (FINNZYMES), and the 0.8 kb DNA fragment from PCR reaction was ligated to a pCRII vector (INVITROGEN) directly. After transformation, plasmids were prepared from an overnight culture of transformed *E. coli* clones, and further analyzed by restriction enzyme digestion and sequencing. The recombinant plasmid containing a sequence encoding the mature PSA was named as pCRII/mPSA.